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(21) International Application Number: PCT/DK96/00498 (22) International Filing Date: 29 November 1996 (29.11.96) (30) Priority Data: 1356/95 30 November 1995 (30.11.95) DK (71) Applicant (for all designated States except US): NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK). (72) Inventors; and (75) Inventors/Applicants (for US only): AASLYNG, Dorrit [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK). SØRENSEN, Niels, Henrik [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK). RØRBÆK, Karen [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK). (74) Common Representative: NOVO NORDISK A/S; Corporate Patents, Novo Allé, DK-2880 Bagsværd (DK).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: AN ENZYME FOR DYING KERATINOUS FIBRES (57) Abstract The present invention relates to a dyeing composition, a method for dyeing keratinous fibres, in particular hair, fur, hide and wool, and the use of a <i>Scytalidium</i> laccase for dyeing.		

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Title: An enzyme for dying keratinous fibres

5 **FIELD OF THE INVENTION**

The present invention relates to a dyeing composition for keratinous fibres, in particular hair, fur, hide and wool, a method for dying and the use of a *Scytalidium* laccase for dyeing.

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BACKGROUND OF THE INVENTION

It has been used for many years to dye the hair to cover appearing grey hair. The need to do so arises from the fact that grey hair is the first sign of having past adolescence, which can be hard to accept for many people.

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For instance, in certain parts of Asia it is widely used by both men and women to dye the hair with dyes often referred to by humorous people as "black shoe polish".

During the last few decades hair dyeing has become more and more popular in the western world. At first Punk Rockers and other society critical groups dyed their hair in extreme colours as a part of their protest against the established society, but today especially many young people also uses hair dyes (in more soft tints than the Punk Rockers) as a sort of "cosmetic" to change or freshen up their "look".

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Hair dyes

In general hair dyeing compositions on the market today can be divided into three main groups:

- 30 - temporary hair dyes,
- semi-permanent hair dyes, and
- permanent oxidative hair dyes.

The temporary hair dyes are only intended to change the natural hair colour for a short period of time and usually functions by depositing dyes on the surface of the hair. Such hair dyes are easy to remove with normal shampooing.

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When using semi-permanent hair dyes the colour of the dyed hair can survive for five or more shampoos. This is achieved

by using dyes having a high affinity for hair keratin and which is able to penetrate into the interior of the hair shaft.

Permanent hair dyes are very durable to sunlight, shampooing and other hair treatments and need only to be refreshed once a month as new hair grows out. With these dyeing systems the dyes are created directly in and on the hair. Small aromatic colourless dye precursors (e.g. p-phenylene-diamine and o-aminophenol) penetrate deep into the hair where said dye precursors are oxidised by an oxidising agent into coloured polymeric compounds. These coloured compounds are larger than the dye precursors and can not be washed out of the hair.

By including compounds referred to as modifiers (or couplers) in the hair dyeing composition a number of hair colour tints can be obtained. Cathecol and Resorcinol are examples of such modifiers.

Traditionally H_2O_2 is used as the oxidizing agent (colour builder), but also as a bleaching agent. Dyeing compositions comprising H_2O_2 are often referred to as "lightening dyes" due to this lightening effect of H_2O_2 .

The use of H_2O_2 in dye compositions have some disadvantages as H_2O_2 damages the hair. Further, oxidative dyeing often demands high pH (normally around pH 9-10), which also inflicts damage on the hair. Consequently, if using dye compositions comprising H_2O_2 it is not recommendable to dye the hair often.

To overcome the disadvantages of using H_2O_2 it has been suggested to use oxidation enzymes to replace H_2O_2 .

US patent no. 3,251,742 (Revlon) describes a method for dyeing human hair by dye formation *in situ* (i.e. on the hair). An oxidative enzyme is used to the colour formation reactions at a substantially neutral pH (pH 7-8.5).

Laccases, tyrosinases, polyphenolases and catacolases are mentioned as the suitable oxidation enzymes.

EP patent no. 504.005 (Perma S.A.) concerns compositions for dying hair which do not require the presence of H_2O_2 (hydrogen peroxide). The composition comprises an enzyme capable of catalyzing the formation of the polymeric dyes and also dye precursors, such as bases and couplers, in a buffer solution wherein the pH of said composition is between 6.5 and 8 and

said enzyme has an optimal activity in the pH range between 6.5 and 8.

Rhizoctonia praticola laccase and *Rhus vernicifera* laccase have a pH-optimum between 6.5 and 8 and can be used to form the polymeric dyes according to this patent.

Abstract of Papers American Chemical Society vol. 209, no. 1-2, 1995 discloses the cloning of a laccase from a *Scytalidium thermophilum*. The abstract does not mention the use of said laccase for dyeing hair.

SUMMARY OF THE INVENTION

The object of the present invention is to provide improved permanent dyeing compositions for keratinous fibres, in particular hair, fur, hide and wool, which is less damaging to the keratinous fibres than e.g. dyeing compositions for hair using H_2O_2 .

It has now surprisingly been found that it is possible to provide such an improved dyeing composition by using an enzyme derived from a strain of the filamentous fungus genus *Scytalidium* as the oxidation enzyme.

In the first aspect the invention relates to a permanent dyeing composition for keratinous fibres, in particular hair, fur, hide and wool, comprising an oxidation enzyme comprising

- 1) one or more oxidation enzymes derived from a strain of the genus *Scytalidium*,
- 2) one or more dye precursors, and
- optionally 3) one or more modifiers.

In a preferred embodiment of the invention the oxidation enzyme is a laccase derived from a strain of the genus *Scytalidium*, in particular from a strain of the species *Scytalidium thermophilum*.

Secondly, it is the object of the invention to provide a method for dyeing keratinous fibres, comprising contacting a laccase derived from a strain of the genus *Scytalidium* with the keratinous fibres and at least one dye precursor in the presence or absence of at least one modifier for a suitable period of time and under conditions sufficient to permit oxidation of the dye precursor into a coloured compound.

Finally the invention relates to the use of an oxidation enzyme derived from a strain of the genus *Scytalidium* for oxidative dyeing of keratinous fibres, in particular hair, fur, hide and wool.

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BRIEF DESCRIPTION OF THE DRAWING

Figure 1 shows the dyeing effect of the *Scytalidium thermophilum* laccase (rStL-FXu-1)

10 DETAILED DESCRIPTION OF THE INVENTION

The object of the present invention is to provide improved permanent dyeing compositions for keratinous fibres, in particular hair, fur, hide and wool, which is less damaging to the keratinous fibres than e.g. hair dyeing compositions using H_2O_2 .

15 It has surprisingly be found that it is possible to provide such an improved dyeing composition by using an oxidation enzyme derived from a strain of the filamentous fungus genus *Scytalidium*.

When using said oxidation enzyme derived from a strain of the genus *Scytalidium* the colour developed is as wash stable as oxidative dyeing of e.g. hair using H_2O_2 and the light fastness is as good as when dyeing chemically.

Consequently, in the first aspect the present invention relates to a permanent dye composition for keratinous fibres, in particular hair, fur, hide and wool, comprising

- 1) one or more oxidation enzymes derived from a strain of the genus *Scytalidium*,
- 2) one or more dye precursors, and
- optionally 3) one or more modifiers.

30 In an embodiment of the invention the oxidation enzyme is a laccase derived from a strain of genus *Scytalidium*, such as a strain of *Scytalidium thermophilum* e.g. the purified laccase described in WO 95/33837 (PCT/US95/06816) from Novo Nordisk, which is hereby incorporated. SEQ ID No 1 shows a DNA sequence
35 encoding a suitable laccase derivable from a strain of the species *Scytalidium thermophilum*.

E. coli JM101 containing the expression vector pShTh15 comprising SEQ ID NO 1 has been deposited under the Budapest

Treaty with the Agricultural Research Service Patent Culture Collection, Northern Regional Research Center, 1815 University Street, Peoria, Illinois, 61604. The vector have been given the Accession Number NRRL B-21262.

- 5 Also contemplated according to the invention are laccases derived from other microorganisms being more than 80% homologous to SEQ ID NO 1 derived from a strain of the species *Scytalidium thermophilum*.

10 In addition, *Scytalidium* laccases also encompass alternative forms of laccases which may be found in *S. thermophilum* and as well as laccases which may be found in other fungi which are synonyms of fall within the definition of *S. thermophilum* as defined by Straatsma and Samson, (1993), Mycol. Res. 97, 321-328). These include *S. indonesiacum*, *Torula thermophila*, *Humicola brevis* var. *thermoidea*, *Humicola brevispora*, *H. grisea* var. *thermoidea*, *Humicola insolens*, and *Humicola lanuginosa* (also known as *Thermomyces lanuginosus*).

15 It is to be understood that the *Scytalidium* laccase may be produced homologously, or heterologously using filamentous fungus, yeast or bacteria as the host cell.

20 Examples of filamentous fungi host cells include strains of the species of *Trichoderma*, preferably a strain of *Trichoderma harzianum* or *Trichoderma reesei*, or a species of *Aspergillus*, most preferably *Aspergillus oryzae* or *Aspergillus niger*, or yeast cells, such as e.g. a strain of *Saccharomyces*, in particular *Saccharomyces cerevisiae*, *Saccharomyces kluyveri* or *Saccharomyces uvarum*, a strain of *Schizosaccharomyces* sp., such as *Schizosaccharomyces pombe*, a strain of *Hansenula* sp., *Pichia* sp., *Yarrowia* sp., such as *Yarrowia lipolytica*, or *Kluyveromyces* sp., such as *Kluyveromyces lactis*, or a bacteria, such as gram-positive bacteria such as strains of *Bacillus*, such as strains of *B. subtilis*, *B. licheniformis*, *B. lentus*, *B. brevis*, *B. stearothermophilus*, *B. alkalophilus*, *B. amyloliquefaciens*, *B. coagulans*, *B. circulans*, *B. lautus*, *B. megaterium* or *B. thuringiensis*, or strains of *Streptomyces*, such as *S. lividans* or *S. murinus*, or gram-negative bacteria such as *Escherichia coli*.

Laccases (benzenediol:oxygen oxidoreductases) (E.C. class

1.10.3.2 according to Enzyme Nomenclature (1992) Academic Press, Inc) are multi-copper containing enzymes that catalyze the oxidation of phenols. Laccase-mediated oxidations result in the production of aryloxy-radical intermediates from suitable phenolic substrates; the ultimate coupling of the intermediates so produced provides a combination of dimeric, oligomeric, and polymeric reaction products. Certain reaction products can be used to form dyes suitable for dyeing hair (see below).

In an embodiment of the invention the *Scytalidium* laccase is neutral. In the context of laccases of the present invention this means that the pH optimum lies in the range from between 6.0 and 8.0.

To obtain dyeing of the keratinous fibres, such as hair, the dyeing composition of the invention also comprises a dye precursor which is converted into a coloured compound (i.e. a dye) by the oxidation agent which according to the invention is an oxidation enzyme derived from a strain of the species *Scytalidium*, such as a strain of *Scytalidium thermophilum*.

Without being limited thereto the dye precursor(s) may be (an) aromatic compound(s) belonging to one of three major chemical families: the diamines, aminophenols (or aminonaphtols) and the phenols. Examples of isatin derivative dye precursors can be found in DE 4,314,317-A1. Further, a number of indole or indoline derivative dye precursors are disclosed in WO 94/00100. Said dye precursors mentioned in these documents are hereby incorporated herein by reference.

Examples of such suitable dye precursors include compounds from the group comprising p-phenylene-diamine (pPD), p-tolylene-diamine, chloro-p-phenylenediamine, p-aminophenol, o-aminophenol and 3,4-diaminotoluene, 2-methyl-1,4-diaminobenzene, 4-methyl-o-phenylenediamine, 2-methoxy-p-phenylenediamine, 2-chloro-1,4-diamino-benzene, 4-amino diphenylamine, 1-amino-4- β -methoxyethylamino-benzene, 1-amino-4-bis-(β -hydroxyethyl)-aminobenzene, 1-3-diamino-benzene, 2-methyl-1,3-diamino-benzene, 2,4-diaminotoluene, 2,6-diaminopyridine, 1-hydroxy-2-amino-benzene, 1-hydroxy-3-amino-benzene, 1-methyl-2-hydroxy-4-amino-benzene, 1-methyl-2-hydroxy-4- β -hydroxyethylamino-benzene, 1-

hydroxy-4-amino-ebnzene, 1-hydroxy-4-methylamino-benzene, 1-methoxy-2,4-diamino-benzene, 1-ethoxy-2,3-diamino-benzene, 1- β -hydroxyethyloxy-2,4-diamino-benzene, phenazines, such as 4,7-phenazinedicarboxylic acid, 2,7-phenazinedicarboxylic acid, 2-phenazinecarboxylic acid, 2,7-diaminophenazine, 2,8-diaminophenazine, 2,7-diamino-3,8-dimethoxyphenazine, 2,7-diamino-3-methoxyphenazine, 3-dimethyl 2,8-phenazinediamine, 2,2'-[(8-amino-7-methyl-2-phenazinyl)imino]bis-ethanol, 2,2'-[(8-amino-7-methoxy-2-phenazinyl)imino]bis-ethanol, 2,2'-[(8-amino-7-chloro-2-phenazinyl)imino]bis-ethanol, 2-[(8-amino-7-methyl-2-phenazinyl)amino]-ethanol, 2,2'-[(8-amino-2-phenazinyl)imino]bis-ethanol, 3-amino-7-(dimethylamino)-2,8-dimethyl-5-phenyl-chloride, 9-(diethylamino)-benzo[a]phenazine-1,5-diol, N-[8-(diethylamino)-2-phenazinyl]-methanesulfonamide, N-(8-methoxy-2-phenazinyl)-Methanesulfonamide, N,N,N',N'-tetramethyl-2,7-phenazinediamine, 3,7-dimethyl-2-phenazinamine, p-amino benzoic acids, such as p-amino benzoic acid ethyl, p-amino benzoic acid glycerid, p-amino benzoic acid isobutyl, p-dimethylamino benzoic acid amil, p-dimethylamino benzoic acid octyl, p-diethoxy amino benzoic acid amil, p-dipropoxy amino benzoic acid ethyl, acetylsalicylic acid, isatin derivatives, such as 2,3-diamino benzoic acid.

In an embodiment the laccase is used with the dye precursor directly to oxidise it into a coloured compound. The dye precursor may be used alone or in combination with other dye precursors.

However, it is believed that when using a diamine or an aminophenol as the dye precursor at least one of the intermediate in the copolymerisation must be an *ortho*- or *para*-diamine or aminophenol. Examples of such are described in US patent no. 3,251,742 (Revlon), the contents of which are incorporated herein by reference.

Optionally the dyeing composition of the invention (especially hair dyeing compositions) also comprises a modifier (coupler) by which a number of colour tints can be obtained. In general modifiers are used in hair dyeing compositions, as the colours resulting from hair dyeing compositions without modifier(s) are usually unacceptable for most people.

Modifiers are typically m-diamines, m-aminophenols, or polyphenols. The modifier (coupler) reacts with the dye precursor(s) in the presence of the oxidative enzyme, converting it into a coloured compound.

5 Examples of modifiers (couplers) include m-phenylenediamine, 2,4-diaminoanisole, 1-hydroxynaphthalene (α -naphthol), 1,4-dihydroxybenzene (hydroquinone), 1,5-dihydroxynaphthalene, 1,2-dihydroxybenzene (pyrocatechol), 1,3-dihydroxybenzene (resorcinol), 1,3-dihydroxy-2-methylbenzene, 1,3-dihydroxy-4-chlorobenzene (4-chlororesorcinol), 1,2,3-trihydroxybenzene, 10 1,2,4-trihydroxybenzene, 1,2,4-trihydroxy-5-methylbenzene, 1,2,4-trihydroxytoluene.

In the second aspect the invention relates to a method for dyeing keratinous fibres, in particular hair, fur, hide and 15 wool, comprising contacting a laccase derived from a strain of the genus *Scytalidium* with the keratinous fibres and at least one dye precursor in the presence or absence of at least one modifier, for a period of time and under conditions sufficient to permit oxidation of the dye precursor into coloured 20 compounds (i.e. a dye).

The dyeing method can be conducted with one or more dye precursors, either alone or in combination with one or more modifiers.

The amount of dye precursor(s) and other ingredients used in 25 the composition of the invention are in accordance with usual commercial amounts.

When using a *Scytalidium* laccase, such as the *Scytalidium thermophilum* laccase mentioned above, the method for dyeing keratinous fibres of the invention may be carried out at room 30 temperature, preferably around the optimum temperature of the enzyme, at a pH in the range from 3.0 to 9.0, preferably 4.0 to 8.0, especially pH 6.0 to 8.0.

Suitable dye precursors and optional modifiers are described above.

35 The use of this *Scytalidium* laccase is an improvement over the more traditional use of H_2O_2 as the latter can damage the keratinous fibres, such as hair. Further, normally prior art methods requires a high pH, which is also damaging to the

keratinous fibres. In contrast hereto, the reaction with laccase can be conducted at acidic or neutral pH, and the oxygen needed for oxidation comes from the air, rather than via harsh chemical oxidation.

- 5 The result provided by the use of the *Scytalidium* laccase is comparable to that achieved with use of H_2O_2 , not only in colour development, but also in wash stability and light fastness. An additional commercial advantage is that a single container package can be made containing both the laccase and the precursor, in an oxygen free atmosphere, which arrangement is not possible with the use of H_2O_2 .

MATERIALS AND METHODS

Materials:

15 Hair:

6" De Meo Virgin Natural White Hair (De Meo Brothers Inc. US)

Enzymes:

- Laccase from *Scytalidium thermophilum* described in
20 WO 95/33837 (PCT/US95/06816) from Novo Nordisk

Deposit of Biological Material

- The following biological material has been deposited on the 25th May 1994 under the terms of the Budapest Treaty with the
25 Agricultural Research Service Patent Culture Collection, Northern Regional Research Center, 1815 University Street, Peoria, Illinois, 61604 and given the following accession number.

- | | |
|---|------------------|
| 30 Deposit | Accession Number |
| <i>E. coli</i> JM101 containing pShTh15 | NRRL B-21262. |

Dye precursors:

- 0.1 % w/w p-phenylene-diamine in 0.1 M K-phosphate buffer, pH
35 7.0. (pPD)
0.1 % w/w p-toluylene-diamine in 0.1 M K-phosphate buffer, pH
7.0.
0.1 % w/w chloro-p-phenylenediamine in 0.1 M K-phosphate

buffer, pH 7.0.

0.1 % w/w p-aminophenol in 0.1 M K-phosphate buffer, pH 7.0.

0.1 % w/w o-aminophenol in 0.1 M K-phosphate buffer, pH 7.0.

5 0.1 % w/w 3,4-diaminotoluene in 0.1 M K-phosphate, buffer pH 7.0.

Modifiers:

0.1 % w/w m-phenylene-diamine in 0.1 M K-phosphate buffer, pH 7.0.

10 0.1 % w/w 2,4-diaminoanisole in 0.1 M K-phosphate buffer, pH 7.0.

0.1 % w/w a-naphthol in 0.1 M K-phosphate buffer, pH 7.0.

0.1 % w/w hydroquinone in 0.1 M K-phosphate buffer, pH 7.0.

0.1 % w/w pyrocatechol in 0.1 M K-phosphate buffer, pH 7.0.

15 0.1% w/w resorcinol in 0.1 M K-phosphate buffer, pH 7.0.

0.1 % w/w 4-chlororesorcinol in 0.1 M K-phosphate buffer, pH 7.0.

20 The dye precursor is combined with one of the above indicated modifiers so that the final concentration in the dyeing solution is 0.1 % w/w with respect to precursor and 0.1 % w/w with respect to modifier.

Other solutions:

25 3% H₂O₂ (in the final dye solution)

Commercial shampoo

Equipment:

Minolta CR200 Chroma Meter

30 Day light bulb: 1000 LUX (D65)

Determination of Laccase Activity (LACU)

35 Laccase activity is determined from the oxidation of syringaldazin under aerobic conditions. The violet colour produced is photometered at 530 nm. The analytical conditions are 19 mM syringaldazin, 23.2 mM acetate buffer, pH 5.5, 30°C, 1 min. reaction time.

1 laccase unit (LACU) is the amount of enzyme that catalyses

the conversion of 1.0 micromole syringaldazin per minute at these conditions.

Assessment of the hair colour

- 5 The quantitative colour of the hair tresses are determined on a Minolta CR200 Chroma Meter by the use the parameters L^* ("0"=black and "100"=white), a^* ("-"=green and "+"=red) and b^* ("-" blue and "+" yellow).
- 10 DL^* , Da^* and Db^* are the delta values of L^* , a^* and b^* respectively compared to L^* , a^* and b^* of untreated hair (e.g. $DL^* = L^*_{\text{sample}} - L^*_{\text{untreated hair}}$).
- 15 DE^* is calculated as $DE^* = \sqrt{DL^{*2} + Da^{*2} + Db^{*2}}$ and is an expression for the total quantitative colour change.

EXAMPLES

Example 1

20

Dyeing effect

The dyeing effect of a *Scytalidium thermophilum* laccase was tested using the dye precursor o-aminophenol and the modifier m-phenylenediamine.

25

Hair dyeing

1 gram De Meo white hair tresses were used.

- 30 4 ml dye precursor solution (including modifier) is mixed with 1 ml laccase on a Whirley mixer, applied to the hair tresses and incubated at 30°C for 60 minutes.

The hair tresses are then rinsed with running water, washed with shampoo, rinsed with running water, combed, and air dried.

The a^* , b^* and L^* was determined on the Chroma Meter and the DE^* values were then calculated.

- 35 A hair tress sample treated without enzyme was used as a blind.

The result of the hair dyeing test is shown in figure 1.

Example 2Wash stability

5 Tresses of white De Meo hair (1 gram) is used for testing the wash stability of hair dyed using *Scytalidium thermophilum* laccase, compared with hair dyed using H_2O_2 , and p-phenylene-diamine (pPD) as the dye precursor. Further the wash stability is compared with a commercial oxidative dye.

10 The oxidative hair dyeing is carried out as described in Example 1.

Hair wash

15 The dyed hair tresses are wetted and washed for 15 seconds with 50 ml of commercial shampoo, and rinsed with water for 1 minute and air dried. The hair tresses are washed up to 18 times.

The a^* , b^* and L^* is determined on the Chroma Meter and the ΔE^* values are then calculated.

20 Example 3

The light fastness

25 Tresses of blond European hair are used for testing the light fastness of hair dyed using *Scytalidium thermophilum* laccase in comparison to hair dyed using H_2O_2 . p-phenylene-diamine was used as dye precursor.

The dyeing of the hair was carried out as described in Example 1.

30 One hair tress is kept dark, while an other is kept at day light (i.e. under a day light bulb (D65)), at approximately 1000 LUX) for up to 275 hours.

The a^* , b^* and L^* parameters are determined immediately after the dyeing of the hair, and further during exposure to day light.

35 ΔE^* then calculated from the determined a^* , b^* and L^* values.

13

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- 5 (i) APPLICANT:
 (A) NAME: Novo Nordisk A/S
 (B) STREET: Novo Alle
 (C) CITY: Bagsvaerd
 (D) COUNTRY: Denmark
 10 (E) POSTAL CODE (ZIP): DK-2880
 (F) TELEPHONE: +45 4444 8888
 (G) TELEFAX: +45 4449 3256
- (ii) TITLE OF INVENTION: An enzyme for dying hair
- 15 (iii) NUMBER OF SEQUENCES: 2
- (v) COMPUTER READABLE FORM:
 (A) MEDIUM TYPE: Floppy disk
 (B) COMPUTER: IBM PC compatible
 20 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

- 25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 2476 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 30 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Scytalidium thermophilum*
- 35 (ix) FEATURE:
 (A) NAME/KEY: intron
 (B) LOCATION: 349..411
- 40 (ix) FEATURE:
 (A) NAME/KEY: intron
 (B) LOCATION: 502..559
- (ix) FEATURE:
 45 (A) NAME/KEY: intron
 (B) LOCATION: 632..686
- (ix) FEATURE:
 50 (A) NAME/KEY: intron
 (B) LOCATION: 1739..1804
- (ix) FEATURE:
 55 (A) NAME/KEY: CDS
 (B) LOCATION: join (106..348, 412..501, 560..631, 687..1738,
 1805..2194)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

60	CTGAATTTAA ATACAGGAAG ATCGCATTCA ATCCAGCCTA GACTGCACAA TGGTTCTGCA	60
	CGACCGTCCG ACACCTGCCA ATAGTGTTAA TAACGGNCTA ATACC ATG AAG CGC TTC	117
	Met Lys Arg Phe	
	1	
65	TTC ATT AAT AGC CTT CTG CTT CTC GCA GGG CTC CTC AAC TCA GGG GCC	165
	Phe Ile Asn Ser Leu Leu Leu Ala Gly Leu Leu Asn Ser Gly Ala	
	5 10 15 20	
	CTC GCG GCT CCG TCT ACA CAT CCC AGA TCA AAC CCC GAC ATA CTG CTT	213

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	Leu	Ala	Ala	Pro	Ser	Thr	His	Pro	Arg	Ser	Asn	Pro	Asp	Ile	Leu	Leu	
					25					30					35		
5	GAA	AGA	GAT	GAC	CAC	TCC	CTT	ACG	TCT	CGG	CAA	GGT	AGC	TGT	CAT	TCT	261
	Glu	Arg	Asp	Asp	His	Ser	Leu	Thr	Ser	Arg	Gln	Gly	Ser	Cys	His	Ser	
				40					45					50			
10	CCA	AGC	AAC	CGC	GCC	TGT	TGG	TGC	TCT	GGC	TTC	GAT	ATC	AAC	ACG	GAT	309
	Pro	Ser	Asn	Arg	Ala	Cys	Trp	Cys	Ser	Gly	Phe	Asp	Ile	Asn	Thr	Asp	
			55					60					65				
15	TAT	GAG	ACC	AAG	ACT	CCA	AAC	ACC	GGA	GTG	GTG	CGG	CGG	GTTAGTATCC			358
	Tyr	Glu	Thr	Lys	Thr	Pro	Asn	Thr	Gly	Val	Val	Arg	Arg				
	70						75					80					
20	CAAGTTACGT	TTGACCAAGA	AATGGACGTG	AAGTGTGCTG	ACTCTCCCGC	TAG											411
	TAC	ACC	TTT	GAT	ATC	ACC	GAA	GTC	GAC	AAC	CGC	CCC	GGT	CCC	GAT	GGG	459
	Tyr	Thr	Phe	Asp	Ile	Thr	Glu	Val	Asp	Asn	Arg	Pro	Gly	Pro	Asp	Gly	
				85					90					95			
25	GTC	ATC	AAG	GAG	AAG	CTC	ATG	CTT	ATC	AAC	GAC	AAA	CTC	CTG	GTAGG		506
	Val	Ile	Lys	Glu	Lys	Leu	Met	Leu	Ile	Asn	Asp	Lys	Leu	Leu			
			100					105					110				
30	GTCCTCTCGA	ACGCCTGCGT	CTGCCACACA	GCGTAAAACT	AACGAACCGC	TAG											559
	GGC	CCG	ACA	GTC	TTC	GCA	AAC	TGG	GGC	GAC	ACC	ATC	GAG	GTG	ACC	GTC	607
	Gly	Pro	Thr	Val	Phe	Ala	Asn	Trp	Gly	Asp	Thr	Ile	Glu	Val	Thr	Val	
				115					120					125			
35	AAC	AAC	CAC	CTG	AGA	ACC	AAC	GGA	GTAAGCGTTC	GGACACAAAG	CCCAGCAACC						661
	Asn	Asn	His	Leu	Arg	Thr	Asn	Gly									
			130					135									
40	TAGACACACT	CAACTGACCA	AGTAG	ACC	TCC	ATC	CAC	TGG	CAC	GGC	TTG	CAC	CAA				716
				Thr	Ser	Ile	His	Trp	His	Gly	Leu	His	Gln				145
								140									
45	AAA	GGA	ACC	AAC	TAC	CAC	GAC	GGC	GCC	AAC	GGC	GTG	ACC	GAG	TGT	CCC	764
	Lys	Gly	Thr	Asn	Tyr	His	Asp	Gly	Ala	Asn	Gly	Val	Thr	Glu	Cys	Pro	
				150					155						160		
50	ATC	CCG	CCC	GGT	GGC	TCC	CGA	GTC	TAC	AGC	TTC	CGA	GCG	CGC	CAA	TAT	812
	Ile	Pro	Pro	Gly	Gly	Ser	Arg	Val	Tyr	Ser	Phe	Arg	Ala	Arg	Gln	Tyr	
				165					170					175			
55	GGA	ACG	TCA	TGG	TAC	CAC	TCC	CAC	TTC	TCC	GCC	CAG	TAT	GGC	AAC	GGC	860
	Gly	Thr	Ser	Trp	Tyr	His	Ser	His	Phe	Ser	Ala	Gln	Tyr	Gly	Asn	Gly	
			180					185					190				
60	GTG	AGC	GGC	GCC	ATC	CAG	ATC	AAC	GGA	CCC	GCC	TCC	CTG	CCC	TAC	GAC	908
	Val	Ser	Gly	Ala	Ile	Gln	Ile	Asn	Gly	Pro	Ala	Ser	Leu	Pro	Tyr	Asp	
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	Ile	Asp	Leu	Gly	Val	Leu	Pro	Leu	Xaa	Asp	Trp	Tyr	Tyr	Lys	Ser	Ala	
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70	GAC	CAG	CTC	GTC	ATC	GAG	ACC	CTG	GCC	AAG	GGC	AAC	GCT	CCG	TTC	AGC	1004
	Asp	Gln	Leu	Val	Ile	Glu	Thr	Leu	Xaa	Lys	Gly	Asn	Ala	Pro	Phe	Ser	
						230			235						240		
75	GAC	AAC	GTC	CTC	ATC	AAC	GGC	ACC	GCA	AAG	CAC	CCC	ACC	ACT	GGC	GAA	1052
	Asp	Asn	Val	Leu	Ile	Asn	Gly	Thr	Ala	Lys	His	Pro	Thr	Thr	Gly	Glu	
				245					250					255			
80	GGG	GAG	TAC	GCC	ATC	GTG	AAG	CTC	ACC	CCG	GGC	AAA	CGC	CAT	CGC	CTG	1100
	Gly	Glu	Tyr	Ala	Ile	Val	Lys	Leu	Thr	Pro	Asp	Lys	Arg	His	Arg	Leu	

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	260	265	270	
5	CGG CTC ATC AAC ATG TCG GTG GAG AAC CAC TTC CAG GTC TCG CTG GCG Arg Leu Ile Asn Met Ser Val Glu Asn His Phe Gln Val Ser Leu Ala 275 280 285			1148
10	AAG CAC ACC ATG ACG GTC ATC GCG GCG GAC ATG GTC CCC GTC AAC GCC Lys His Thr Met Thr Val Ile Ala Ala Asp Met Val Pro Val Asn Ala 290 295 300 305			1196
15	ATG ACC GTC GAC AGC CTG TTT ATG GCC GNC GGG CAG CGG TAT GAT GTT Met Thr Val Asp Ser Leu Phe Met Ala Val Gly Gln Arg Tyr Asp Val 310 315 320			1244
20	ACC ATC GAC GCG AGC CAG GCG GTG GGG AAT TAC TGG TTC AAC ATC ACC Thr Ile Asp Ala Ser Gln Ala Val Gly Asn Tyr Trp Phe Asn Ile Thr 325 330 335			1292
25	TTT GGA GGG CAG CAG AAG TGC GGC TTC TCG CAC AAT CCG GCG CCG GCA Phe Gly Gly Gln Gln Lys Cys Gly Phe Ser His Asn Pro Ala Pro Ala 340 345 350			1340
30	GCC ATC TTT CGC TAC GAG GGC GCT CCT GAC GCT CTG CCG ACG GAT CCT Ala Ile Phe Arg Tyr Glu Gly Ala Pro Asp Ala Leu Pro Thr Asp Pro 355 360 365			1388
35	GGC GCT GCG CCA AAG GAT CAT CAG TGC CTG GAC ACT TTG GAT CTT TCA Gly Ala Ala Pro Lys Asp His Gln Cys Leu Asp Thr Leu Asp Leu Ser 370 375 380 385			1436
40	CCG GTG GTG CAA AAG AAC GTG CCG GTT GAC GGG TTC GTC AAA GAG CCT Pro Val Val Gln Lys Asn Val Pro Val Asp Gly Phe Val Lys Glu Pro 390 395 400			1484
45	GGC AAT ACG CTG CCG GTG ACG CTC CAT GTT GAC CAG GCC GCG GCT CCA Gly Asn Thr Leu Pro Val Thr Leu His Val Asp Gln Ala Ala Pro 405 410 415			1532
50	CAC GTG TTT ACG TGG AAG ATC AAC GGG AGC GCT GCG GAC GTG GAC TGG His Val Phe Thr Trp Lys Ile Asn Gly Ser Ala Ala Asp Val Asp Trp 420 425 430			1580
55	GAC AGG CCG GTG CTG GAG TAT GTC ATG AAC AAT GAC CTG TCT AGC ATT Asp Arg Pro Val Leu Glu Tyr Val Met Asn Asn Asp Leu Ser Ser Ile 435 440 445			1628
60	CCG GTC AAG AAC AAC ATT GTG AGG GTG GAC GGA GTC AAC GAG TGG ACG Pro Val Lys Asn Asn Ile Val Arg Val Asp Gly Val Asn Glu Trp Thr 450 455 460 465			1676
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80	TCC CCC GAC GTC TCG CCC GAT TCA GAA ACC CGC TTC GTC TTT GAC CCG Ser Pro Asp Val Ser Pro Asp Ser Glu Thr Arg Phe Val Phe Asp Pro 490 495 500			1879
85	GCC GTC GAC CTC CCC CGT CTG CGC GGA CAC AAC CCC GTC CGG CGC GAC Ala Val Asp Leu Pro Arg Leu Arg Gly His Asn Pro Val Arg Arg Asp 505 510 515			1927

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5	GTC ACC ATG CTT CCC GCG CGC GGC TGG CTG CTG CTG GCC TTC CGC ACG Val Thr Met Leu Pro Ala Arg Glu Trp Leu Leu Leu Ala Phe Arg Thr 520 525 530	1975
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15	TCG GGC GGG TTA AGC GTC GAC TTT CTG GAG CGG CCG GAC GAG CTG CGC Ser Gly Gly Leu Ser Val Asp Phe Leu Glu Arg Pro Asp Glu Leu Arg 550 555 560 565	2071
20	GGG CAG CTG ACG GGA GAG AGC AAG GCG GAG TTG GAG CGT GTT TGT CGC Gly Gln Leu Thr Gly Glu Ser Lys Ala Glu Leu Glu Arg Val Cys Arg 570 575 580	2119
25	GAG TGG AAG GAT TGG GAG GCG AAG AGC CCG CAT GGG AAG ATC GAT TCG Glu Trp Lys Asp Trp Glu Ala Lys Ser Pro His Gly Lys Ile Asp Ser 585 590 595	2167
30	GGG TTG AAG CAG CGG CGA TGG GAT GCG TGAGGTAGTT GGGCGGATTG Gly Leu Lys Gln Arg Arg Trp Asp Ala 600 605	2214
35	TTTAACACGT AGTGGGTAAG GTTGGGGCGG GTTTGTTTGG CGTTTTCAGG GGTGGGGTG CGGATGCTGG TCATCCGGGA AACGGCTCTA CAACTGGTGT CAATAGACTA ATATAGAGTG ATCAAAGAAC TGAGGTTCTG AAAGAGGCGT GGAAGTCGCG TTGTGACTCC CTTTGCCATG TTGGGAAGTG TGGCTCAACA TTGTGTTTCAG GTTTGCTCAG GGTGATNTCG AACTGACGTN TTGATGAGGG TTATTGCNTA GA	2274 2334 2394 2454 2476

(2) INFORMATION FOR SEQ ID NO: 2:

40	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 616 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
45	(ii) MOLECULE TYPE: protein
50	(vi) ORIGINAL SOURCE: (A) ORGANISM: <i>Scytalidium thermophilum</i>
55	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:
60	Met Lys Arg Phe Phe Ile Asn Ser Leu Leu Leu Ala Gly Leu Leu 1 5 10 15
65	Asn Ser Gly Ala Leu Ala Ala Pro Ser Thr His Pro Arg Ser Asn Pro 20 25 30
70	Asp Ile Leu Leu Glu Arg Asp Asp His Ser Leu Thr Ser Arg Gln Gly 35 40 45
75	Ser Cys His Ser Pro Ser Asn Arg Ala Cys Trp Cys Ser Gly Phe Asp 50 55 60
80	Ile Asn Thr Asp Tyr Glu Thr Lys Thr Pro Asn Thr Gly Val Val Arg 65 70 75 80
85	Arg Tyr Thr Phe Asp Ile Thr Glu Val Asp Asn Arg Pro Gly Pro Asp 85 90 95

17

Gly Val Ile Lys Glu Lys Leu Met Leu Ile Asn Asp Lys Leu Leu Gly
 100 105 110
 5 Pro Thr Val Phe Ala Asn Trp Gly Asp Thr Ile Glu Val Thr Val Asn
 115 120 125
 Asn His Leu Arg Thr Asn Gly Thr Ser Ile His Trp His Gly Leu His
 130 135 140
 10 Gln Lys Gly Thr Asn Tyr His Asp Gly Ala Asn Gly Val Thr Glu Cys
 145 150 155 160
 Pro Ile Pro Pro Gly Gly Ser Arg Val Tyr Ser Phe Arg Ala Arg Gln
 165 170 175
 15 Tyr Gly Thr Ser Trp Tyr His Ser His Phe Ser Ala Gln Tyr Gly Asn
 180 185 190
 20 Gly Val Ser Gly Ala Ile Gln Ile Asn Gly Pro Ala Ser Leu Pro Tyr
 195 200 205
 Asp Ile Asp Leu Gly Val Leu Pro Leu Gln Asp Trp Tyr Tyr Lys Ser
 210 215 220
 25 Ala Asp Gln Leu Val Ile Glu Thr Leu Ala Lys Gly Asn Ala Pro Phe
 225 230 235 240
 Ser Asp Asn Val Leu Ile Asn Gly Thr Ala Lys His Pro Thr Thr Gly
 245 250 255
 30 Glu Gly Glu Tyr Ala Ile Val Lys Leu Thr Pro Asp Lys Arg His Arg
 260 265 270
 35 Leu Arg Leu Ile Asn Met Ser Val Glu Asn His Phe Gln Val Ser Leu
 275 280 285
 Ala Lys His Thr Met Thr Val Ile Ala Ala Asp Met Val Pro Val Asn
 290 295 300
 40 Ala Met Thr Val Asp Ser Leu Phe Met Ala Xaa Gly Gln Arg Tyr Asp
 305 310 315 320
 Val Thr Ile Asp Ala Ser Gln Ala Val Gly Asn Tyr Trp Phe Asn Ile
 325 330 335
 45 Thr Phe Gly Gly Gln Gln Lys Cys Gly Phe Ser His Asn Pro Ala Pro
 340 345 350
 50 Ala Ala Ile Phe Arg Tyr Glu Gly Ala Pro Asp Ala Leu Pro Thr Asp
 355 360 365
 Pro Gly Ala Ala Pro Lys Asp His Gln Cys Leu Asp Thr Leu Asp Leu
 370 375 380
 55 Ser Pro Val Val Gln Lys Asn Val Pro Val Asp Gly Phe Val Lys Glu
 385 390 395 400
 Pro Gly Asn Thr Leu Pro Val Thr Leu His Val Asp Gln Ala Ala Ala
 405 410 415
 60 Pro His Val Phe Thr Trp Lys Ile Asn Gly Ser Ala Ala Asp Val Asp
 420 425 430
 65 Trp Asp Arg Pro Val Leu Glu Tyr Val Met Asn Asn Asp Leu Ser Ser
 435 440 445
 Ile Pro Val Lys Asn Asn Ile Val Arg Val Asp Gly Val Asn Glu Trp
 450 455 460

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	465					470					475					480
5	His	Pro	Met	His	Leu	His	Gly	His	Asp	Phe	Phe	Val	Leu	Gly	Arg	Ser
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10	Val	Asp	Leu	Pro	Arg	Leu	Arg	Gly	His	Asn	Pro	Val	Arg	Arg	Asp	Val
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15	Thr	Met	Leu	Pro	Ala	Arg	Gly	Trp	Leu	Leu	Leu	Ala	Phe	Arg	Thr	Asp
		530					535					540				
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20	Gly	Gly	Leu	Ser	Val	Asp	Phe	Leu	Glu	Arg	Pro	Asp	Glu	Leu	Arg	Gly
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	Gln	Leu	Thr	Gly	Glu	Ser	Lys	Ala	Glu	Leu	Glu	Arg	Val	Cys	Arg	Glu
				580					585					590		
25	Trp	Lys	Asp	Trp	Glu	Ala	Lys	Ser	Pro	His	Gly	Lys	Ile	Asp	Ser	Gly
			595					600					605			
30	Leu	Lys	Gln	Arg	Arg	Trp	Asp	Ala								
		610					615									

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13 bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>9</u> , line <u>21-31</u>	
B. IDENTIFICATION OF Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depository institution Agricultural Research Service Patent Culture Collection (NRRL)	
Address of depository institution (including postal code and country) Northern Regional Research Center 1815 University Street Peoria, IL 61604, US	
Date of deposit 25 May 1994	Accession Number NRRL B-21262
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
In respect of those designations in which a European and/or Australia Patent is sought, during the pendency of the patent application, a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (Rule 28(4) EPC/Regulation 3.25 of Australia Statutory Rule 1991 No. 71).	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indication listed below will be submitted to the International Bureau Later (specify the general nature of the indications e.g. "Accession Number of Deposit")	

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PATENT CLAIMS

1. A dyeing composition comprising an oxidation enzyme characterised in that the composition comprises:

- 5 1) one or more oxidation enzymes derived from a strain of the genus *Scytalidium*,
2) one or more dye precursors, and
optionally 3) one or more modifiers.

2. The dyeing composition according to claim 1, wherein the
10 oxidation enzyme is derived from a strain of the genus *Scytalidium* laccase

3. The dyeing composition according to claim 2, wherein the laccase is derived from a strain of the species *Scytalidium thermophilum*.

15 4. The dyeing composition according to claims 2 and 3, wherein the laccase is neutral.

5. The dyeing composition according to claim 3, having the sequence shown in SEQ ID No 1.

6. The dyeing composition according to claim 5, wherein the
20 sequence encoding the laccase is homologous to the SEQ ID NO 1.

7. The dyeing composition according to claim 6, wherein the sequence encoding the laccase is more than 80% homologous to SEQ ID NO 1.

8. The dyeing composition according to any of claims 1 to 7,
25 comprising a dye precursor selected from the group comprising p-phenylene-diamine (PPD), p-toluylene-diamine, chloro-p-phenylenediamine, p-aminophenol, o-aminophenol and 3,4-diaminotoluene, 2-methyl-1,4-diaminobenzene, 4-methyl-o-phenylenediamine, 2-methoxy-p-phenylenediamine, 2-chloro-1,4-diamino-benzene,
30 4-amino diphenylamine, 1-amino-4- β -methoxyethylamino-benzene, 1-amino-4-bis-(β -hydroxyethyl)-amonibenzene, 1-3-diamino-benzene, 2-methyl-1,3-diamino-benzene, 2,4-diaminotoluene, 2,6-diaminopyridine, 1-hydroxy-2-amino-benzene, 1-hydroxy-3-amino-benzene, 1-methyl-2-hydroxy-4-amino-benzene, 1-methyl-2-hydroxy-4- β -hydroxyethylamino-benzene, 1-hydroxy-4-amino-benzene, 1-hydroxy-4-methylamino-benzene, 1-methoxy-2,4-diamino-benzene, 1-ethoxy-2,3-diamino-benzene, 1- β -hydroxyethyloxy-2,4-diamino-

35

benzene, phenazines, such as 4,7-phenazinedicarboxylic acid, 2,7-phenazinedicarboxylic acid, 2-phenazinecarboxylic acid, 2,7-diaminophenazine, 2,8-diaminophenazine, 2,7-diamino-3,8-dimethoxyphenazine, 2,7-diamino-3-methoxyphenazine, 2,7-diamino
 5 3-methoxyphenazine, 3-dimethyl 2,8-phenazinediamine, 2,2'-[(8-amino-7-methyl-2-phenazinyl)imino]bis-ethanol, 2,2'-[(8-amino-7-methoxy-2-phenazinyl)imino]bis-ethanol, 2,2'-[(8-amino-7-chloro-2-phenazinyl)imino]bis-ethanol, 2-[(8-amino-7-methyl-2-phenazinyl)amino]-ethanol,
 10 2,2'-[(8-amino-2-phenazinyl)imino]bis-ethanol, 3-amino-7-(dimethylamino)-2,8-dimethyl-5-phenyl-chloride, 9-(diethylamino)-benzo[a]phenazine-1,5-diol, N-[8-(diethylamino)-2-phenazinyl]-methanesulfonamide, N-(8-methoxy-2-phenazinyl)-Methanesulfonamide, N,N,N',N'-tetramethyl-2,7-phenazinediamine, 3,7-dimethyl-2-phenazinamine, p-
 15 amino benzoic acids, such as p-amino benzoic acid ethyl, p-amino benzoic acid glycerid, p-amino benzoic acid isobutyl, p-dimethylamino benzoic acid amil, p-dimethylamino benzoic acid octyl, p-diethoxy amino benzoic amil, p-dipropoxy amino benzoic acid ethyl, acetylsalicylic acid, isatin derivatives,
 20 such as 2,3-diamino benzoic acid.

9. The dyeing composition according to claims 8, comprising a dye modifier selected from the group comprising m-phenylenediamine, 2,4-diaminoanisole, 1-hydroxynaphthalene(α -naphthol), 1,4-dihydroxybenzene(hydroquinone), 1,5-dihydroxynaphthalene,
 25 1,2-dihydroxybenzene(pyrocatechol), 1,3-dihydroxybenzene (resorcinol), 1,3-dihydroxy-2-methylbenzene, 1,3-dihydroxy-4-chlorobenzene (4-chlororesorcinol), 1,2,3-trihydroxybenzene, 1,2,4-trihydroxybenzene, 1,2,4-trihydroxy-5-methylbenzene, 1,2,4-trihydroxytoluene.

30 10. A method for dyeing comprising contacting a laccase derived from a strain of the genus *Scytalidium* with the keratinous fibres and at least one dye precursor in the presence or absence of at least one modifier for a period of time and under conditions sufficient to permit oxidation of the dye precursor
 35 into a coloured compound.

11. The method according to claim 10, wherein the dyeing is carried out at a pH in the range from 3.0 to 9.0, preferably 4.0 to 8.0, especially 6.0 to 8.0.

12. Use of an oxidation enzyme derived from a strain of the genus *Scytalidium* for oxidative dyeing keratinous fibres, in particular hair, fur, hide and wool.

13. The use according to claim 14, wherein the oxidation
5 enzyme is derived from a strain of the species *Scytalidium thermophilum*.

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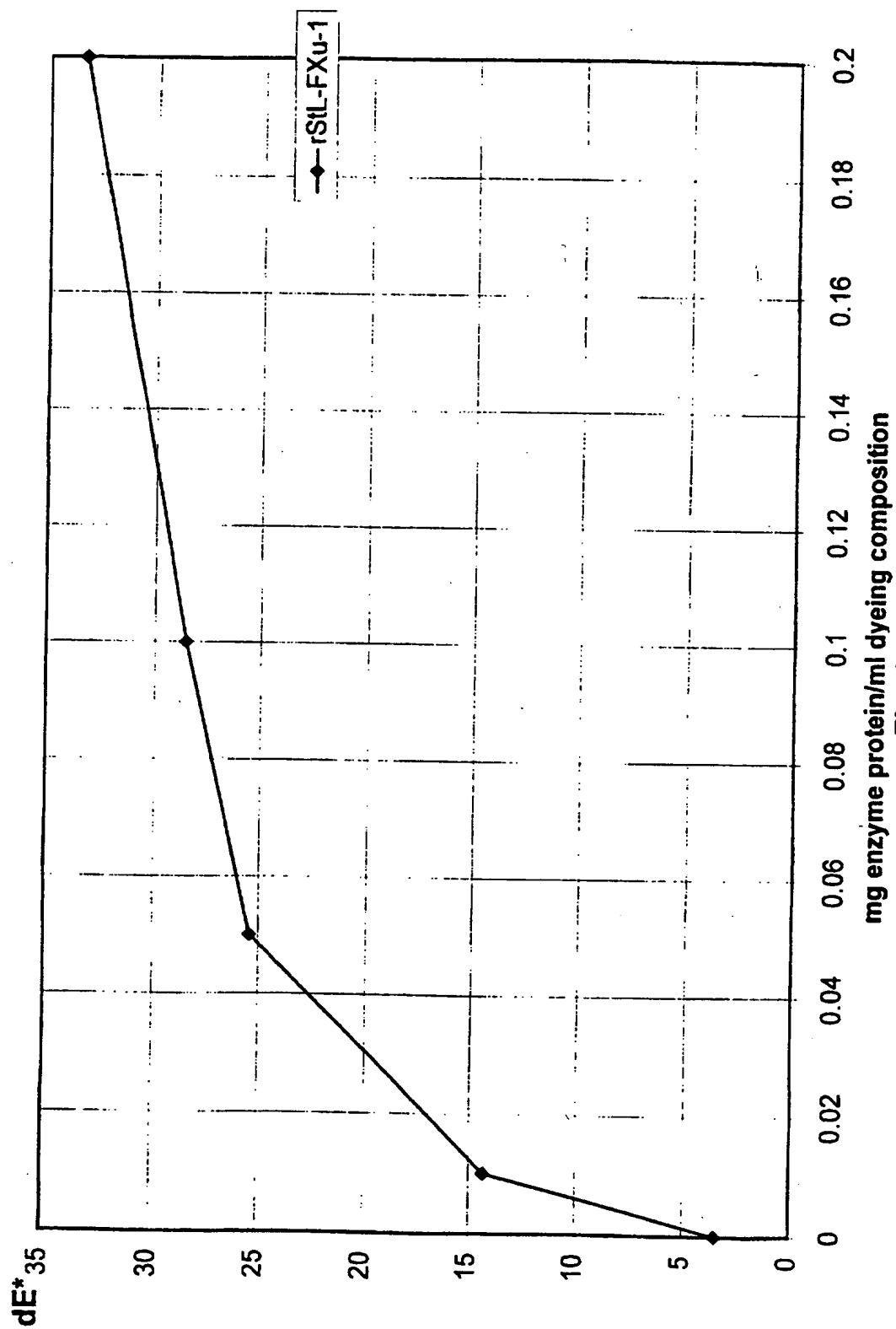


Fig. 1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 96/00498

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C09B 67/00, A61K 7/13

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C09B, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 9533837 A1 (NOVO NORDISK BIOTECH, INC.), 14 December 1995 (14.12.95), claims 28, 29; page 15, line 34 - page 16 --	1-13
P,A	WO 9533836 A1 (NOVO NORDISK BIOTECH, INC.), 14 December 1995 (14.12.95), claims 31-42; page 16, line 12 - page 17, line 27; page 34, line 20 - page 36 --	1-13
X	EP 0504005 A1 (PERMA SOCIETE ANONYME), 16 Sept 1992 (16.09.92) --	1-13

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

28 February 1997

Date of mailing of the international search report

01-03-1997

Name and mailing address of the ISA/

Swedish Patent Office

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Authorized officer

Gerd Strandell

Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 96/00498

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 3251742 A (SAUL SOLOWAY), 17 May 1966 (17.05.66) --	1-13
X	WO 9600290 A1 (NOVO NORDISK BIOTECH, INC.), 4 January 1996 (04.01.96), claims 37-48; page 48, line 25 - page 54, line 24 --	1-13
X	STN International, File CAPLUS, CAPLUS accession no. 1991:498981, Saruno, Rinjiro: "Hair- dyeing preparations containing melanin or other polyphenol pigments and manufacture of the pigments"; & JP, A2, 910403 --	1-13
X	STN International, File CAPLUS, CAPLUS accession no. 1995:974547, Chivukula, Muralikrishna et al: "Phenolic azo dye oxidation by laccase from Pyri- cularia oryzae"; & Appl. Environ. Microbiol. (1995), 61(12), 4374-77 --	1-13
A	DE 4314317 A1 (HENKEL KGAA), 3 November 1994 (03.11.94) --	8
A	WO 9400100 A1 (L'OREAL), 6 January 1994 (06.01.94) --	8
A	WO 9507988 A1 (NOVO NORDISK A/S), 23 March 1995 (23.03.95), claim 41 -- -----	1-13

INTERNATIONAL SEARCH REPORT

Information on patent family members

03/02/97

International application No.

PCT/DK 96/00498

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 9533837	14/12/95	AU-A- 2656695	04/01/96
WO-A1- 9533836	14/12/95	AU-A- 2656595	04/01/96
EP-A1- 0504005	16/09/92	AT-T- 121931	15/05/95
		CA-A- 2061826	09/09/92
		DE-D, T- 69202290	09/11/95
		ES-T- 2072720	16/07/95
		FR-A, B- 2673534	11/09/92
		JP-A- 6172145	21/06/94
US-A- 3251742	17/05/66	FR-A- 1363462	00/00/00
		GB-A- 993923	00/00/00
WO-A1- 9600290	04/01/96	AU-A- 2827895	19/01/96
DE-A1- 4314317	03/11/94	EP-A- 0695162	07/02/96
		JP-T- 8509478	08/10/96
		WO-A- 9424988	10/11/94
WO-A1- 9400100	06/01/94	DE-D, T- 69301464	05/06/96
		EP-A, B- 0645999	05/04/95
		FR-A, B- 2692782	31/12/93
		JP-T- 7508271	14/09/95
		US-A- 5538517	23/07/96
WO-A1- 9507988	23/03/95	AU-A- 7833694	03/04/95
		CA-A- 2171288	23/03/95
		CN-A- 1133067	09/10/96
		EP-A- 0719337	03/07/96
		FI-A- 961250	18/03/96
		US-A- 5480801	02/01/96